

## SPATIAL DISTRIBUTION OF SOME ECTOMYCORRHIZAL FUNGI (RUSSULACEAE, FUNGI, BASIDIOMYCOTA) IN FOREST HABITATS FROM THE NORTH-EAST REGION (ROMANIA)

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**Abstract:** Ectomycorrhizal macromycetes are, generally, an important ecological component for forest habitats, and a valuable resource in the context of sustainable development of rural communities in the North-East Region of Romania. The woody species distribution is an extremely important factor for the ECM macromycetes presence. The purpose of this study was to elaborate maps of potential distribution for some ECM edible macromycetes from *Russula* and *Lactarius* genera, based on chorological information, ICAS Forest Types Map, vegetation tables and bibliographical sources. These information allowed the elaboration of 15 potential maps of distribution for 15 edible species of *Russula* and *Lactarius*. The study was based entirely on the plant – fungal associations. The results highlighted that in the North-East Region of Romania there is a noteworthy potential for *Russulaceae* species. As expected, there is a large amplitude of species presence in the field depending on the fungal specificity for tree host and tree species distribution.

**Keywords:** ECM, *Lactarius*, *Russula*, North-East Region, potential distribution map.

### Introduction

There are approximately 100000 known species of fungi on Earth [BOA, 2004] and many have economic value [BOA, 2004]. More than 3000 species possess some degree of edibility and are used in kitchens across world [GARIBAY-ORIJEL, 2009]. In temperate zones, many edible fungi are ectomycorrhizal (ECM). ECM fungi have a special relationship with plants, being in symbiosis with them [TĂNASE & MITITIUC, 2001]. Extensions of radicular system of plants, ECM fungi absorb nutrients and water from soil and give these resources to the plant [MARTINOVÁ & al. 2015; PEŠKOVA & al. 2012; WOLFE & PRINGLE, 2012]. On the other way, the plant supplies fungi the carbohydrates and other organic compounds necessary for development [TĂNASE & MITITIUC, 2001]. Plant diversity and composition is influenced by mycorrhizae [PEAY & al. 2009] and across forest ecosystems, fungi play a vital functional role [BUÉE & al. 2011]. On the other side, ECM fungi fruit bodies apparition is influenced especially by the host phenology [BUNTGEN, 2013]. Any change in the forest ecosystem can drive important phenology shifts, especially if the disturbance is powerful, like those induced by hazards (insect attacks [ȘTURSOVA & al. 2009], fires [KUTORGA & al. 2012], droughts [PEŠKOVA &

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al. 2012], strong storms [MILLER & LODGE, 1997]). In temperate zones, vegetation composition and structure influences fungal diversity and fungal distribution patterns [ANGELINI & al. 2014]. Important plant families with distribution in temperate areas are associated with ECM fungi: *Betulaceae*, *Fagaceae*, *Pinaceae*, *Tiliaceae* [MARTINOVÁ & al. 2015]. Numerous studies confirmed differences between different forest types, in relation to fungal diversity and composition, especially between broad-leaved and coniferous forests [ANGELINI & al. 2014; KUTSEGI & al. 2015]. Therefore, fungal geographical distribution is driven by the type of forests, as well as other related variables like soil upper layer's parameters [PEAY & al. 2009; SHI & al. 2013; MARTINOVÁ & al. 2015; DINCĂ & DINCĂ, 2015; KUTSEGI & al. 2015]. The forest type and its abiotic characteristics can be used as a powerful predictive variable for the macromycetes distribution, in our case from *Russula* and *Lactarius* genera.

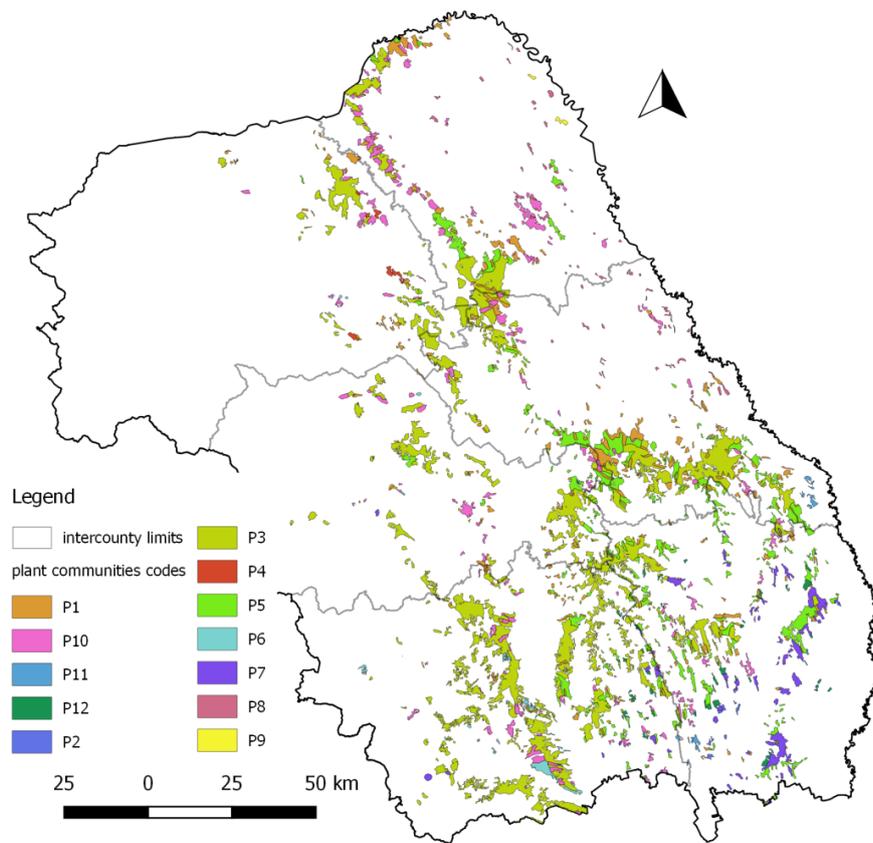
*Lactarius* and *Russula* genera are included in *Russulaceae* family, *Agaricales* order, *Hymenomycetes* class and *Basidiomycotina* phylum. These genera are the main members of the *Russulaceae* family in Europe [ADAMČÍK & al. 2006]. The total number of species is unknown, but the family's diversity is high [ADAMČÍK & al. 2006]. These macromycetes can form mycorrhizae with one tree species (e.g. *Lactarius deliciosus* with *Pinus*, *L. deterrimus* with *Picea*; COURTECUISSÉ & DUHEM, 2013) or with many woody hosts (e.g. *Russula delica* with *Carpinus*, *Fagus*, *Quercus*, *Tilia*; GERHARDT, 1999). There are two different ways of using edible fungi: personal subsistence or marketing [BOA, 2004], and in North-East Region, most of forest collected fungi are used for personal culinary use or for local marketing, *Russula* and *Lactarius* fungi having their part. Numerous studies have also highlighted the effect of some *Russulaceae* species to the good human health [SUN & al. 2010; NANDI & al. 2012; KOSANIĆ & al. 2016; RUTHES & al. 2013; SARIKURKCU & al. 2008]. On the other hand, *Russulaceae* family can prove as an important group for fungal diversity in North-East Region's forests, as PAVEL (2007) found that *Russulaceae* family and *Russula* genus was the most diverse genus in *Quercus* sp. edified forests. The value of *Russula* and *Lactarius* is therefore multiple, many species having commercial value, being important for human health or important from ecological perspective.

The importance of forest can be emphasized (among other important services and products) through their potential for wild edible fungi. The fact that a large part of these fungi are ECM [BOA, 2004] and the *Russulaceae* form an important group in forest's myco-diversity, made us to approach this group in the study. The aims of the study were: (i) to generate maps with common woody hosts across North-East Region; (ii) to generate maps of potential distribution for edible *Russula* and *Lactarius* species in North-East Region's forests; (iii) to prove that any literature source can be useful in primary assessment of potential distribution mapping; (iiii) to show, through mapping, that forests types have a powerful impact on fungi presence.

### Materials and methods

In order to generate potential distribution maps, the first step was to determine the area in which those maps are set to be created. Forest ecosystems distribution across North-East Region of Romania was obtained from the ICAS forest types map (1997). After

clipping the interest region, a number of 76 forest types was obtained. Next, a correspondence between forest types and plant communities was realized using CHIFU & al. (2006). Thus, a distribution map of 12 plant communities in the North-East Region resulted. The *Pino-Quercetum* association, mapped in the south-western part of the region, was excluded because the study's purpose was to select only *Quercus* sp. forests, without coniferous species in their composition. Another reason for *Pino - Quercetum* exclusion was that it covered less than 1% of the total area (Fig. 1).



**Fig. 1.** Distribution map of broad-leaved forests in North-East Region of Romania (adapted after ICAS Forest Types Map, 1997)

The investigated fungal species were selected as having a minimal edibility. Five *Lactarius* species and ten *Russula* species were chosen on this criterion, using literature [TĂNASE & al. 2009; GERHARDT, 1999; COURTECUISSSE & DUHEM, 2013]. For these species, four tree genera were identified as hosts: *Carpinus*, *Fagus*, *Quercus* and *Tilia*.

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Depending on the number of tree - hosts, probability numbers were assigned to fungi – plant mycorrhizae: 0 for no mycorrhizae, 100 for common mycorrhizae and 25 for rarely mycorrhizae (Tab. 2). For each studied tree genus all constancy classes were extracted from CHIFU & al. (2006). The maximum percentage value was chosen as the value for the tree genera in each plant community.

**Tab. 1.** Forest communities with *Quercus* sp. in the North-East Region of Romania

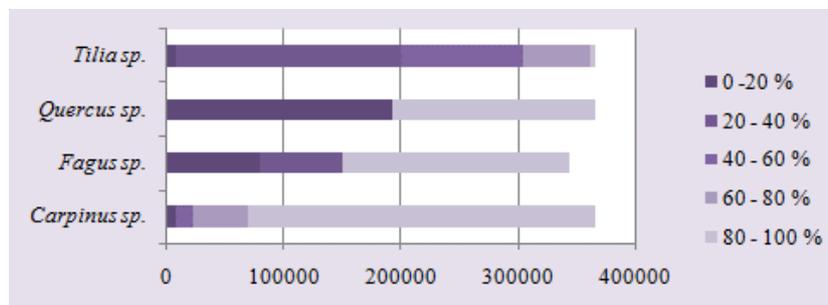
Code	Association	Class	Dominant tree genera (maximum constancy class $\geq 4$ )
P1	<i>Aro orientalis</i> – <i>Carpinetum</i> * <i>quercetosum roboris</i>	<i>Quercu</i> – <i>Fagetea</i>	<i>Carpinus</i> , <i>Quercus</i> , <i>Tilia</i>
P2	<i>Aro orientalis</i> – <i>Carpinetum</i> * <i>quercetosum pedunculiflorae</i>	<i>Quercu</i> – <i>Fagetea</i>	<i>Carpinus</i> , <i>Quercus</i> , <i>Tilia</i>
P3	<i>Lathyro venetus</i> – <i>Fagetum</i> * <i>fagetosum</i>	<i>Quercu</i> – <i>Fagetea</i>	<i>Fagus</i> , <i>Carpinus</i>
P4	<i>Lathyro venetus</i> – <i>Fagetum</i> * <i>quercetosum dalechampii</i>	<i>Quercu</i> – <i>Fagetea</i>	<i>Carpinus</i> , <i>Fagus</i> , <i>Quercus</i>
P5	<i>Dentario quinquefoliae</i> – <i>Carpinetum</i> * <i>typicum</i>	<i>Quercu</i> – <i>Fagetea</i>	<i>Carpinus</i> , <i>Quercus</i>
P6	<i>Genisto tinctoriae</i> – <i>Quercetum</i> <i>petraeae</i>	<i>Quercetea robori</i> – <i>petraeae</i>	<i>Carpinus</i> , <i>Quercus</i>
P7	<i>Tilio tomentosae</i> – <i>Quercetum</i> <i>dalechampii</i> * <i>typicum</i>	<i>Quercetea pubescentis</i>	<i>Quercus</i> , <i>Tilia</i>
P8	<i>Aceri tatarico</i> – <i>Quercetum roboris</i>	<i>Quercetea pubescentis</i>	<i>Quercus</i> , <i>Tilia</i> , <i>Carpinus</i>
P9	Communities with <i>Acer campestre</i> and <i>Quercus robur</i>	<i>Quercetea pubescentis</i>	<i>Quercus</i>
P10	<i>Quercetum robori</i> – <i>petraeae</i>	<i>Quercetea pubescentis</i>	<i>Quercus</i> , <i>Carpinus</i>
P11	<i>Quercetum pedunculiflorae</i>	<i>Quercetea pubescentis</i>	<i>Quercus</i>
P12	<i>Cotino</i> – <i>Quercetum pubescentis</i>	<i>Quercetea pubescentis</i>	<i>Quercus</i>

The next step in map processing consisted in importing the final ecosystem map in R software and transforming map's data to a more appropriate type, the raster format. For each tree genera, the area (in ha) of each forest type per each constancy value was calculated. The total number of pixels, where the rasters resolution was 100 x 100 m (Fig. 2) were considered for this purpose.

After importing the potential distribution map of plant communities in R software, a data frame was generated for further analysis. There was a 30 columns with minimum and maximum probability of occurrence of fungi based on tree – fungi association and tree constancy values. In total, 360 cells. For computing the probability of fungi occurrence, a modified form of the law of total probability was used.

**Tab. 2.** Occurrence probability of selected *Lactarius* and *Russula* species and their tree genera hosts

code	mushroom species	<i>Carpinus</i>	<i>Fagus</i>	<i>Quercus</i>	<i>Tilia</i>
m1	<i>Lactarius pallidus</i>	0	100	25	0
m2	<i>Lactarius piperatus</i>	0	100	100	0
m3	<i>Lactarius quietus</i>	0	0	100	0
m4	<i>Lactarius subdulcis</i>	0	100	0	0
m5	<i>Lactarius vellereus</i>	0	100	25	0
m6	<i>Russula aurea</i>	0	100	25	25
m7	<i>Russula cyanoxantha</i>	0	100	100	0
m8	<i>Russula delica</i>	100	100	100	100
m9	<i>Russula graveolens</i>	25	25	100	0
m10	<i>Russula grisea</i>	100	25	100	0
m11	<i>Russula heterophylla</i>	25	100	100	25
m12	<i>Russula olivacea</i>	0	100	0	0
m13	<i>Russula vesca</i>	25	100	25	0
m14	<i>Russula violeipes</i>	0	100	25	0
m15	<i>Russula virescens</i>	0	100	100	0

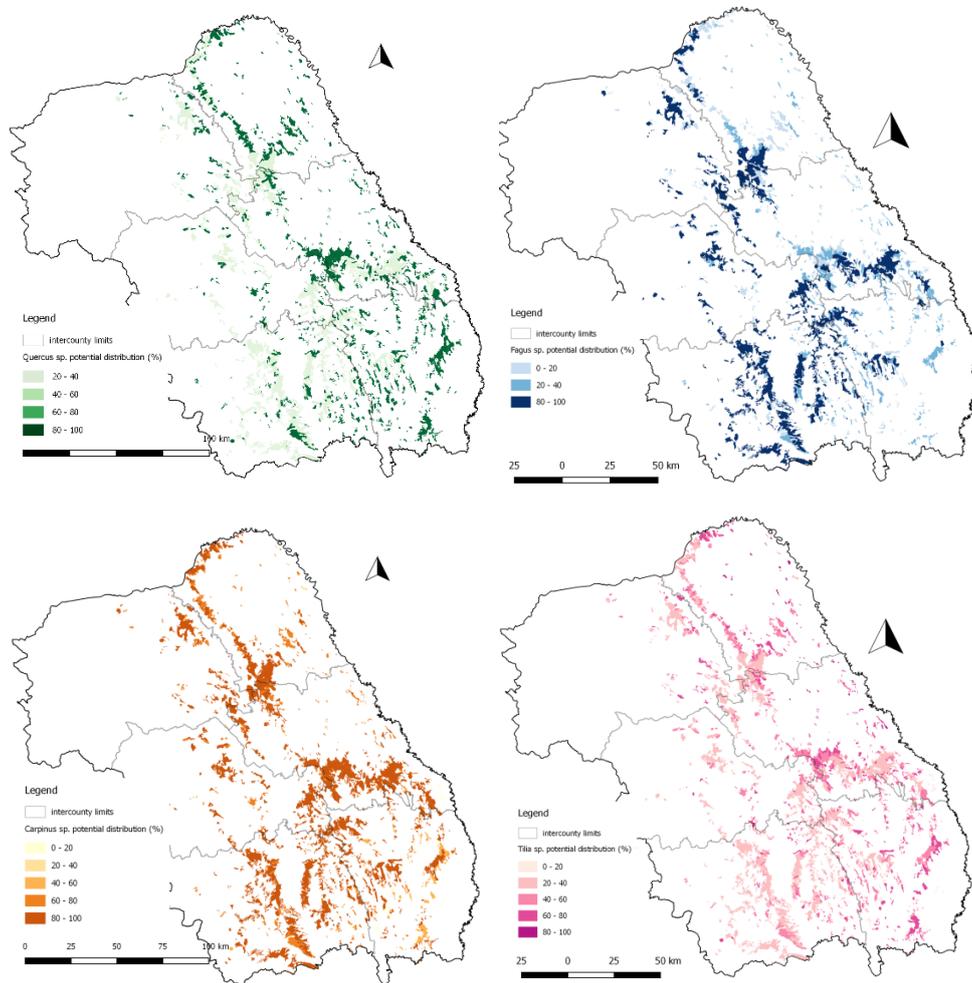


**Fig. 2.** Occurrence probability and occupied area (ha) of tree-hosts genera in the North-Eastern Romania

Suppose that  $B_1, B_2, \dots, B_n$  is a collection of exclusive events, each one with its specific probability space, for any event  $A$ , the probability of  $A$ 's occurrence in the same total space is:

$$P(A) = \sum P(A \cap B_i) = \sum P(A|B_i) \cdot P(B_i) \text{ [ZWILLINGER \& KOKOSKA, 2000].}$$

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**Fig. 3.** *Quercus*, *Fagus*, *Carpinus* and *Tilia* genera potential distribution map (the corresponding colors used are: green, blue, brown and pink)

Because the formula states that, given the total space, only one event  $A_i$  can happen, the formula was adapted so that each event can happen at the same time. In this way, the theorem's limitation that in a given forest type, a fungal species can occur only because of a single association, is eliminated. The fungi probability associations with tree genera were modified according to formula 1:

$$PFm_i(f_k, t_j) = Pm_i(f_k, t_j) * 100 / \sum Pm_i(f_k, t_j).$$

The law of total probability was therefore generated in formula 2:

$$Pf_k(m_i) = \sum Pf_k(t_j) * PFm_i(f_k t_j) / 100,$$

where  $Pf_k(m_i)$  it's the probability that the mushroom species  $m_i$  can be found in polygon  $f_k$ ;  $Pf_k(t_j)$  is the maximum probability that the woody plant genera  $t_j$  can be found in polygon  $f_k$ ;  $PFm_i(f_k t_j)$  is the maximum probability that the fungi species  $m_i$  can be found in polygon  $f_k$  only on available tree genera  $t_j$  while  $PFm_i(f_k t_j)$  have the same interpretation without being transformed through formula 1; where  $j$  is from 1 to 4 and the number of tree genera; where  $i$  is from 1 to 15 and the number of fungi and  $k$  is from 1 to 12 and the number of plant communities. In this formula, were used: the total number of trees genera available in the polygon forest ( $NTtF$ ), the total number of tree genera, 4 ( $NTt4$ ) or the total number of trees available for the mushroom ( $NTtM$ ). For an *in situ* fungi probability of occurrence, only the tree species growing on that site can be considered. Therefore, a fourth variable was taken, the total number of trees that grows on that site and have mycorrhizae with fungi ( $NTtMF$ ). After incorporation of the final matrix in to the shapefile's data, 15 maps with the maximum distribution of the majority of edible *Russula* and *Lactarius* species in North-East Region's forests with *Quercus* sp. (Fig. 5 and Fig. 6a, 6b) were generated.

Another objective of this study was to observe if some groups of forest types could be differentiated by fungi probability occurrence. Thus, an attempt to group forest types depending on fungi probability of occurrence by K-means clustering was carried out. Silhouette index was calculated in order to identify the optimum number of clusters. This was obtained using *fviz\_nbclust* function from *factoextra* package in R software (R Core Development Team, 2015). The data used was composed only from the mushrooms maximum probability occurrence values, in the 12 plant communities. In this case, the optimum number was three clusters (Fig. 4).

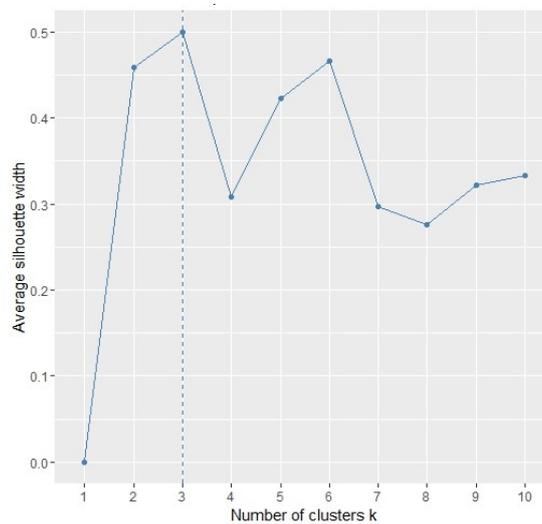
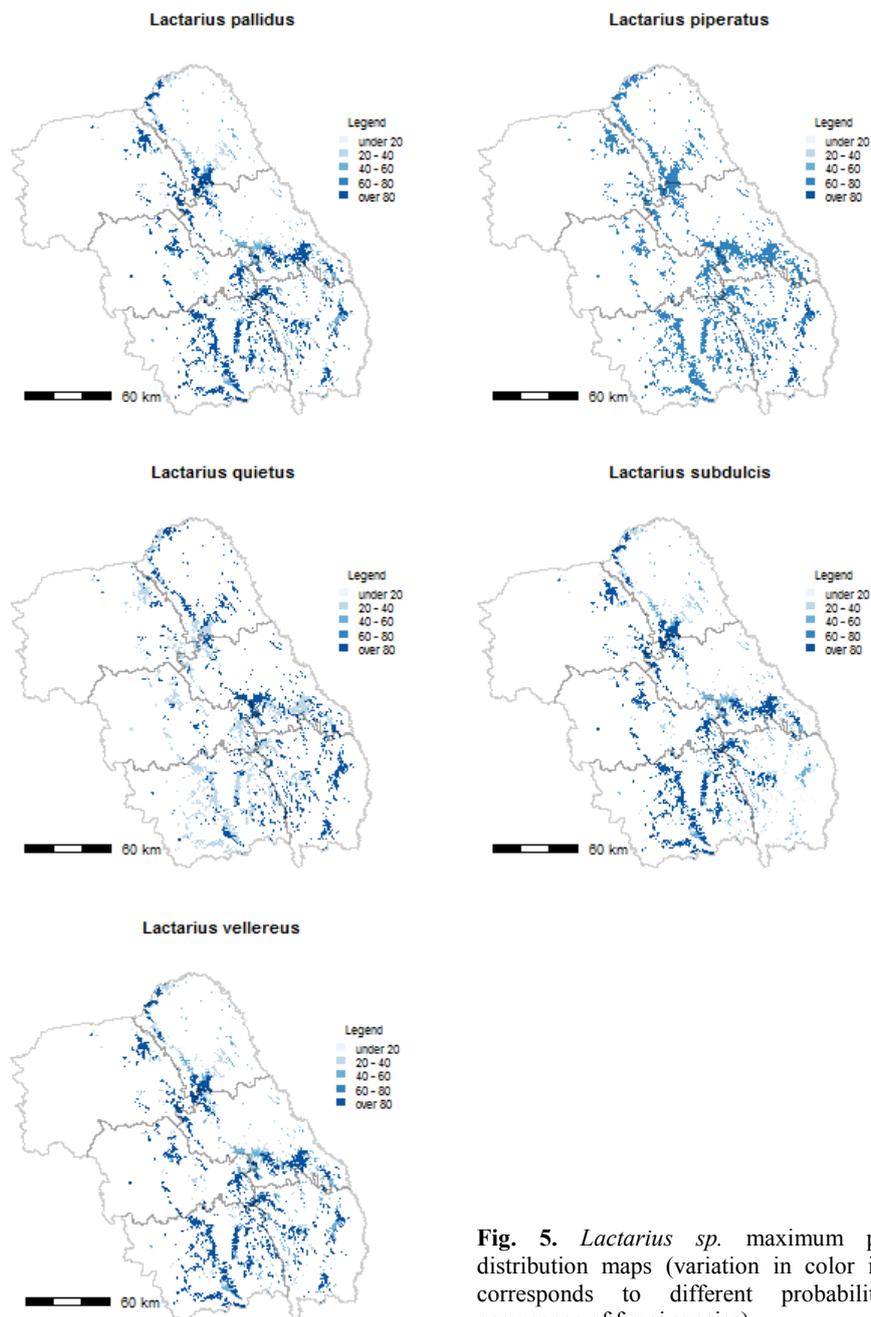
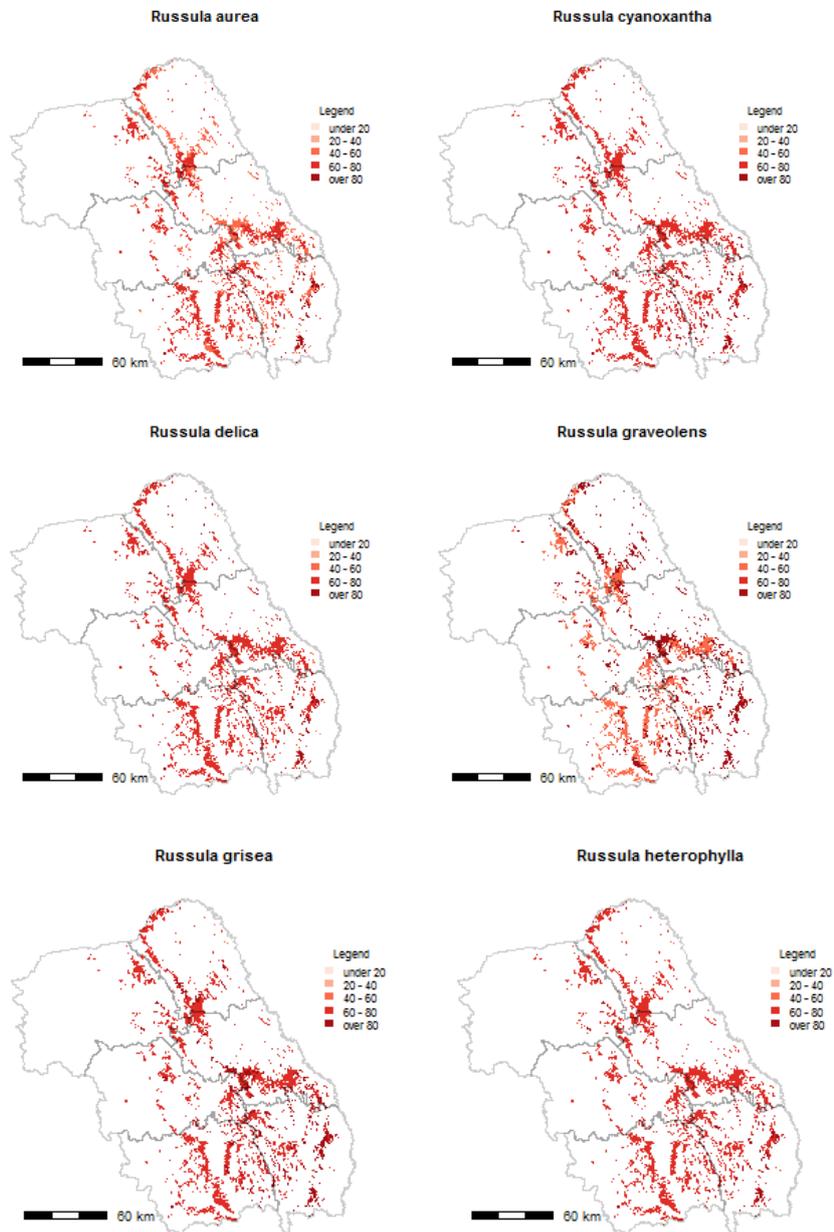


Fig. 4. Identification of optimum number of clusters using the *Silhouette* index

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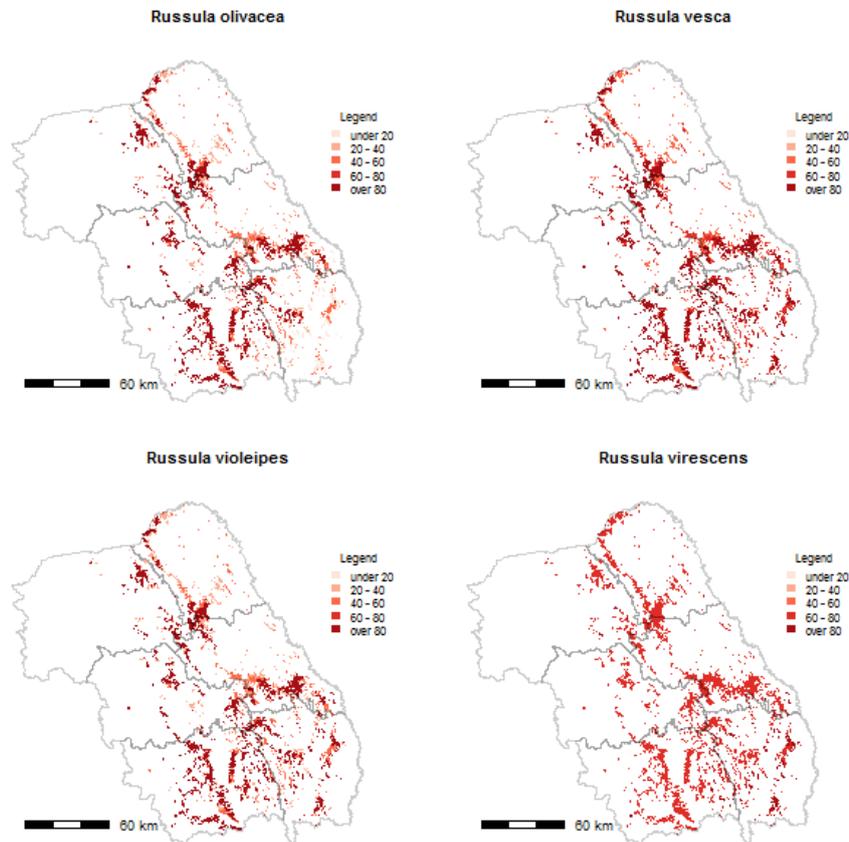


**Fig. 5.** *Lactarius sp.* maximum potential distribution maps (variation in color intensity corresponds to different probabilities of occurrence of fungi species).



**Fig. 6a.** *Russula* sp. maximum potential distribution maps (variation in color intensity corresponds to different probabilities of occurrence of fungi species).

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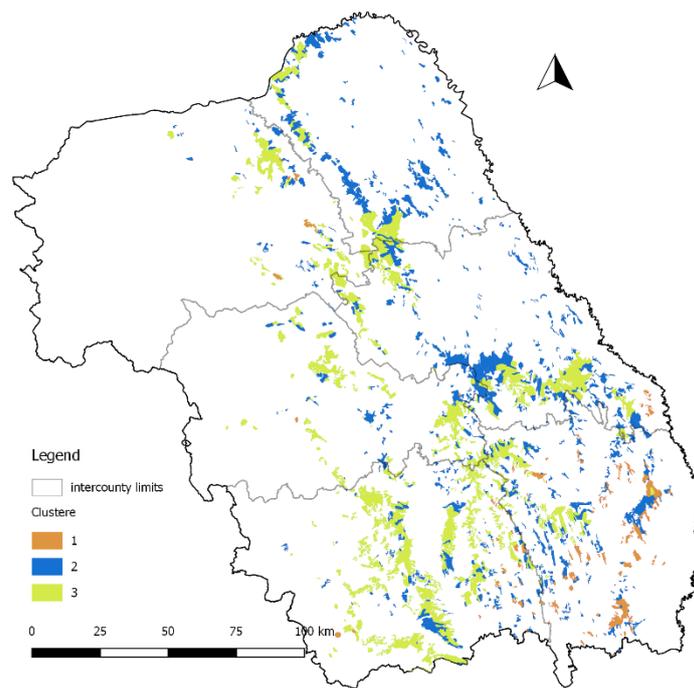
**Fig. 6b.** *Russula sp.* maximum potential distribution maps (variation in color intensity corresponds to different probabilities of occurrence of fungi species)

### Results and discussion

The study methodology consisted in systematic use of the probabilities for fungi and tree species occurrence. This algorithm uses at maximum the plant constancy values and cartographic representations for generation of the potential distribution maps. These generated maps are a product of mathematics and one explanatory variable: fungi preference to particular plant tree species. The data base used was stretched from fungi to woody plant relations and from woody plant presence and constancy in certain plant communities.

A significant difference from a fungal species to another (in respect to probability of occurrence) was observed, due to different host preferences, different total number of hosts and different constancy values of trees in different plant communities. It was also found that there is a similarity of distribution probabilities between fungal species making mycorrhizae with identical or merely identical hosts. According to the maps of fungi

maximum probability of occurrence (Fig. 5 and Fig. 6a, Fig. 6b), the majority of fungi have a large distribution area. The Russulaceae species with lower probability of occurrence in different plant communities were *L. subdulcis* (code *m4*) and *R. olivacea* (code *m12*), because of their affinity only to *Fagus* species. On the other side, *L. quietus* (code *m3*), which form mycorrhizae only with *Quercus* genus, had a larger spectrum of occurrence.



**Fig. 7.** Map of the three groups (resulted in k-means clustering) of forest types aggregated depending on fungi probability of occurrence in North-East Region of Romania.

Comparing the potential area of occurrence for each plant community and each fungal species, it was observed that, for the majority of fungi, association *Lathyro venetus* – *Fagetum \*fagetosum* comprised the largest part of 100% probability of occurrence, about 136315 ha. The next three plant communities were *Dentario quinquefoliae* – *Carpinetum*, *Aro orientalis* – *Carpinetum \*quercetosum roboris* and *Quercetum robori* – *petraeae*, with values between 20300 and 40300 ha. These four plant communities became the most important when considering edible *Russula* and *Lactarius* species in *Quercus* forests of North-East Region of Romania. Appreciated culinary species like *Russula cyanoxantha*, *R. delica* or *R. virescens* had a 100 % presence surface of approximately 235741 – 250097 ha, mostly in the former four plant communities. The species with the largest area of 100% probability occurrence were *R. vesca* and *R. grisea*, with over 260000 ha, both having 3 genera of trees as mycorrhizae partners: *Quercus*, *Fagus*, *Carpinus*.

The first group included *Tilio tomentosae* – *Quercetum dalechampii*, *Quercetum pedunculiflorae*, *Cotino* – *Quercetum pubescentis* and *Lathyro venetus* – *Fagetum* sub-association *quercetosum dalechampii* plant communities, all characterized by high frequency and dominance of *Quercus* species. Excepting the last plant community, all other were characterized by no *Fagus* sp. presence. Most of the studied mushrooms had a high probability of occurrence in this plant communities within this cluster. This shows that *Quercus* species was an important driver for the ECM fungi found in North-East Region forests. The last community was characterized by codominance of both *Fagus* and *Quercus* genera. It was a particular case in all clustered data. In the first 3 plant communities can be observed that the most frequent (and dominant) tree genera (*Quercus* and *Tilia*) were represented by xerophytic species (particularly *Q. pedunculiflora*, *Q. pubescens* and *T. tomentosa*) [SÁRBU & al. 2013]. Mean annual temperature was highlighted as an important driver for ECM distribution [SHI & al. 2013]. In this context, the above mentioned tree species, which are more thermophilous could explain the more restricted spatial distribution (only in the south-eastern part of the study area) of plant communities.

The second group comprised *Aro orientalis* – *Carpinetum*, *Dentario quinquefoliae* – *Carpinetum*, *Aceri tatarico* – *Quercetum roboris*, *Quercetum robori* – *petraeae*, *Genisto tinctoriae* – *Quercetum petraeae* and *Acer campestre* with *Quercus robur* communities. This group was characterized by increased frequencies and dominance of *Quercus* sp., lower values for *Fagus* sp., while the other two tree genera had maximum probabilities of occurrence ranging from 40% to 100%. The last cluster included *Lathyro venetus* – *Fagetum \*fagetosum* and was characterized (distinctively from other plant communities) by *Fagus* sp. dominance and *Quercus* sp. low occurrence probability (maximum of 20%). Also, the cluster groups the only combination of low *Quercus* sp. probability occurrence with high *Carpinus* sp. occurrence probability. Both sub-associations of *Lathyro venetus* – *Fagetum* provides maximum probability of occurrence for mushrooms specialized with *Fagus* genera, as there were, in this study, *Lactarius subdulcis* and *Russula olivacea*. Regarding the differentiation between the second and the third clusters, the fungi that had large amplitude probability of occurrence were *Lactarius quietus*, *L. subdulcis* and *Russula olivacea*, all having only one tree genera host. This showed that, mushrooms affinity for particular tree genera can prove as an important driver for an entire fungal group. As others authors found, dominant tree species is one of the main drivers for shaping fungal communities in temperate forests [GOLDMANN & al. 2015; SHI & al. 2013]. Fungal species having multiple tree hosts, like *Russula delica*, *R. graveolens*, *R. grisea*, *R. heterophylla* and *R. vesca*, could be found in more forest types having at least one tree genera host in its composition. Because of that, at regional scales, some mushrooms can be found in many ecosystem types, from Mediterranean [ANGELINI & al. 2015; AZUL & al. 2011] to temperate forests [BUÉE & al. 2011], as shown for ECM species like *Russula cyanoxantha* or *R. delica*.

Regarding the distribution of these groups (Fig. 7), the clusters 2 and 3 had a large latitudinal and longitudinal amplitude, while the first cluster was preponderantly located in the southern and south-eastern part of the region. Clusters 1 and 2 comprised 7.3%, respectively 33.7% of the total forest area, while *Fagus* sp. dominated clustered had 59.0% of the total forest surface. Therefore, edible *Russula* and *Lactarius* species may prove a particular regional affinity, considering the forests dominant trees. Among the main factors separating the forests was the occurrence probability of *Quercus* sp. (especially clusters 1

and 2 from 3) and of *Fagus* sp. The fact that *Fagus* sp. is an important driver for edible *Lactarius* and *Russula* species could be explained by the fact that the number of these species associations with *Fagus* sp. is far larger (14 vs. 5 and 3) than other codominant species (*Carpinus* sp. and *Tilia* sp.) with *Quercus* sp. Dominant species in phytocenoses covering large areas stimulates diversity of other organism groups [FODOR & al. 2002].

The successful host genera *Quercus* (Tab. 2) in number of associative fungi - 50% from *Russulaceae* family was also demonstrated by RICHARD & al. (2004) who found that *Russula* and *Cortinarius* accounted for 34.4% of ECM fruitbodies and 50% of species diversity. As shown by TOJU & al. (2013), in a *Quercus* sp. temperate Japanese forest, roots colonized by both ECM fungi (including many species of *Lactarius* and *Russula* genera) and root endophytes may explain the complexity of fungal communities in oak dominated forests. Also, deep-rooted tree species like *Quercus robur* and *Q. petraea* [ȘOFLETEA & CURTU, 2007] might enhance survival of ECM fungi during prolonged drought, as shown with other *Quercus* species in California [SMITH & al. 2007]. PAVEL (2007) found more edible and non-edible *Russula* species in *Quercus* sp. edified forests than in *Picea abies* forests, both in Dofteana and Hemeiuș forests. O'HANLON (2011) found that Irish oak forests are the home for as many ECM species as the Irish Scots pine forests are. He found that oak forests hosts with 50% more *Lactarius* species than Scots pine, ash or Sitka spruce forests and has similar numbers in terms of *Russula* species as Scots pine forests. In total, *Russula* and *Lactarius* genera occupied the fourth and fifth places in a ranking fungal genera across all four forests types. Significant variation in composition of fungal communities was found also by GOLDMANN & al. (2015), who analyzed them in a comparative study between beech and coniferous forests. The most important driver which controls the fungal distribution, especially the ECM ones is the plant host [FODOR & al. 2002]. *Russula* and *Lactarius* genera are known to have a wider distribution than other ECM species [GOLDMANN & al. 2015].

### Conclusions

In this study, 15 species with edibility potential included in *Russula* and *Lactarius* genera were investigated, in the forest ecosystems from the North-East Region of Romania, and maps of the potential distribution for each fungal species were generated. There are differences and similarities between distribution maps, determined by the fungal host specificity and host's probability of occurrence in the field. There are fungal species whose distribution maps are projections of the ecosystem edified by the dominant tree - in this case, the genus of tree-host. Significant differences between distribution maps of species preferring broad-leaved forests with *Quercus* sp. in North-East Region were observed.

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